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Commentary:

Deconstructing the winding path to the recapitulation of mammalian oogenesis *ex vivo* or

Perseverance pays off in the *ex vivo* propagation of healthy mammalian oocytes

David F. Albertini and Evelyn Telfer

Producing functioning gametes for investigating the causes of human infertility is perhaps the highest goal on the regenerative medicine agenda. Mammalian gametogenesis in males or females is a complex process wedding the generation of sex cells capable of supporting embryogenesis with events played out within the somatic confines of the testis or ovary. The path towards the completion of oogenesis requires a longstanding collaboration between somatic cells of the ovarian follicle that first engage primordial germ cells as they enter into meiotic prophase. These newly formed oocytes come to rest within the primordial follicle pool to be siphoned off later during the adult reproductive lifespan. In this issue, Morohaku and colleagues deploy an elegant series of experimental manipulations that convincingly demonstrate how primordial germ cells in mice can be propagated through all stages of oogenesis and result in the production of healthy living offspring(1). On the pathway to deconstructing and reconstructing the pathway through oogenesis *ex vivo*, these investigations uncover several surprising details that will inform future studies aimed at defining the mechanisms underlying oogenesis and long awaited potential clinical applications.

The road to their success begins in 1996 with studies coming from the Eppig laboratory establishing that functional oocytes could be developed from *in vitro* growth of primordial follicles (2) . Further refinements in their methodology improved efficiency for collection of differentiated oocytes (3). Importantly, work from the Eppig laboratory demonstrated the utility of reconstituting mouse ovarian somatic and germ cells in culture with eventual transplant experiments documenting the pluripotent capacity to generate a mammalian ovary capable of supporting follicle development (4). But until publication of the Morohaku paper, all efforts to recapitulate oogenesis *ex vivo* have required complementation by tissue transplantation with limited success.

An important clue came from the studies of Hayashi and colleagues in 2012(5). After coaxing mouse embryonic stem cells (ES) or induced pluripotent stem cells (iPSC) into a primordial germ cell-like state,

they showed an age/stage-dependent requirement of the ovarian soma in order for the transplanted cells to become integrated into follicles capable of ovulating and producing oocytes with fertility potential, as shown by the birth of live young. These studies and others emphasize the critical nature of an ongoing dialogue between ovarian somatic cells that invokes a panoply of signaling mechanisms necessary to establish and maintain oocyte/follicle development to its ultimate state, ovulation (6) (7). Indications from previous genetic manipulation studies identified key paracrine effectors at the interface between oocytes and somatic cells(8), but the extent to which these cues would be self-generating and operative in an *in vitro* culture system appeared to be problematic given the enormous complexity at specific stages (6) and the many extrinsic factors now known to influence the dialogue *in vivo*(9). A closer look at the pathway taken by Morohaku et al., suggests otherwise.

Beginning with fetal 12.5 dpc ovaries, their initial studies hit an immediate roadblock when it was discovered that abnormal follicles emerged after 7-10 days of culture maintained in fetal bovine serum (FBS) but not an artificial serum substitute (SPS). Systematically narrowing the search for culprits led them to estradiol17B (E2) such that inclusion of an estrogen receptor antagonist in the culture medium encouraged sustained development of follicles whose enclosed oocytes proceeded through a hypertrophic growth phase only slightly less well than age-matched *in vivo* controls. From a technical perspective, two more obstacles were awaiting resolution that would be tested not only by morphological signs of normal development but would rely heavily on data obtained via RNA-seq serving the role of a GPS system guiding each step forward. So it was that inclusion of follicle-stimulating hormone (FSH) and 2% PVP as a cell interaction stabilizer between days 17 and 20 supported further growth and development and a whiff of collagenase over the last two weeks of culture (29-43 days in total) resulted in a formulation yielding healthy oocytes demonstrated to produce live young following *in vitro* fertilization and embryo transfer with conventional technology (Figure 1).

What distinguishes this work from previous attempts to recapitulate oogenesis *in vitro* is not only the perseverance and attention to detail at each step along the road to fruition- live young-but the evaluation of oocyte quality parameters known from studies of mouse oogenesis *in vivo* to be critical for production of healthy oocytes. For example, this group long ago recognized that the competence to acquire and exercise completion of the process of meiosis took place during the growth phase of oogenesis and required epigenetic modifications in oocyte chromatin (10). Keep in mind that the processes of growth and transcriptional efficiency coupled to histone modifications required for the expression of housekeeping and maternal effect gene products (11) involve dynamic histone changes that precede imprinting methylations (12) . Moreover, these stage specific methylation marks, of both imprinted and nonimprinted varieties, control later developmental events bearing directly on implantation and trophectoderm development (13). Again, the paper by Mokoharu et al validates appropriate epigenetic modifications along the road to propagating the oocytes they document will give rise to healthy offspring.

In many ways this paper reinforces the utility and intrinsic plasticity of the mouse model for future studies into the underpinnings of gametogenesis in the female as shown in previous investigations(14). But whether it sets the stage for moving *in vitro* gametogenesis into the realm of practical application in other mammalian species or clinical applications remains questionable (15). Any extrapolation to the

human must be weighed against the significant differences in oocyte and follicle competence reported as a function of age (16) as well as many major differences between mouse and human ovaries over the spectrum that is folliculogenesis (17, 18).

In leaving no stone unturned, Morohaku and colleagues proffer an interesting twist towards the end of their report having to do with the feasibility of using cryopreserved ovaries and asking whether their system for propagating oocytes is applicable. Unsurprisingly, they show it is possible to generate developmentally competent oocytes from frozen fetal ovaries, albeit with less efficiency. With the field of fertility preservation steadily moving in the direction of artificial devices that could support oogenesis from primordial germ cells or stem cells (19), the clues provided from these studies on mice define a point of departure for future work aimed at accomplishing what Morokaru and colleagues have contributed to the field of in vitro gametogenesis.

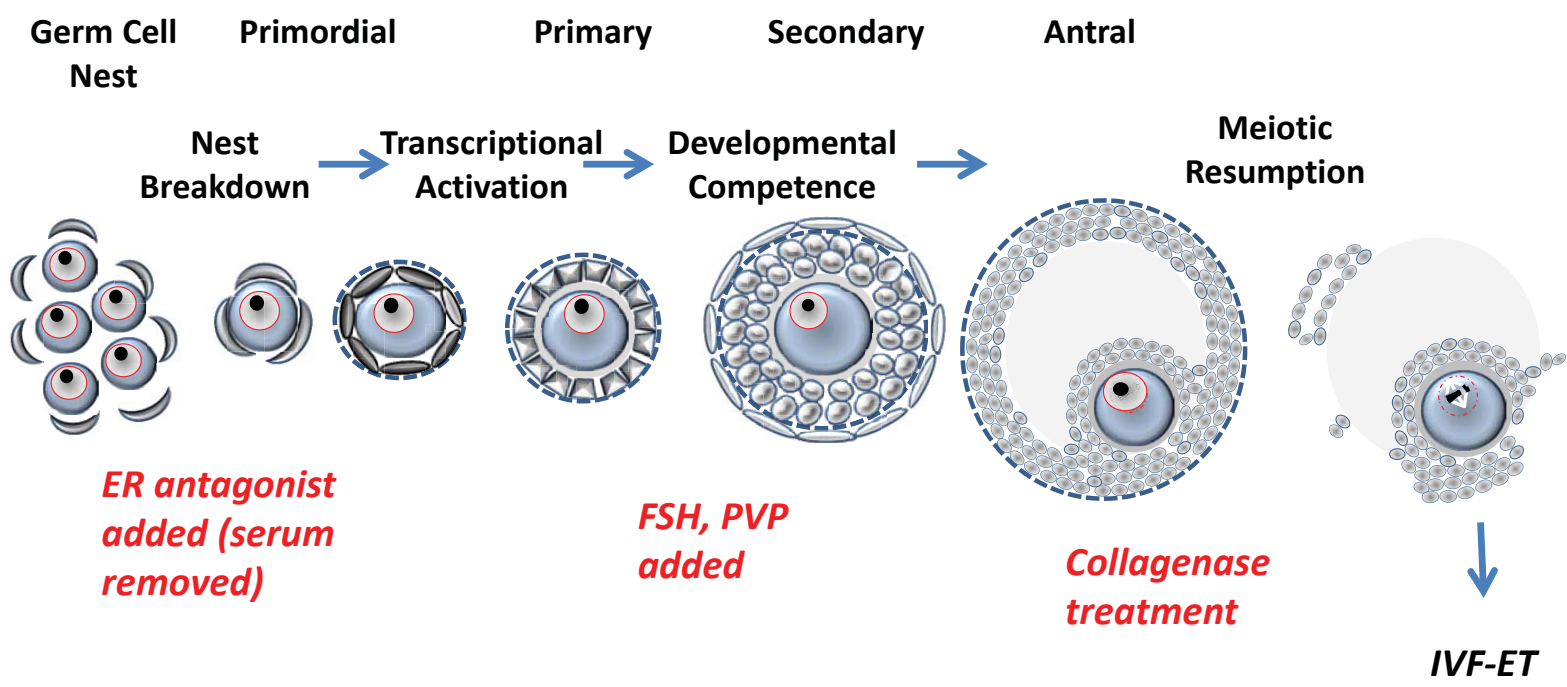
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Figure 1. Schematic summarizing the experimental system devised by Morohaky *et al* to obtain developmentally competent mouse oocytes in vitro. Main stages of follicle growth are shown at the top highlighting major transition events as they would occur during ovary development in vivo. Essential modifications to culture media are shown in red at the times corresponding to day of culture (blue table, bottom). Corresponding stage of in vivo ovarian development is shown below the time of culture.



Day 0	Day 5-11	Day 12-16	Day 17-20	Day 21-29	Day 29-34	Day 34-43
12.5dpc	birth	5do	10do	14 do	21 do	22-36 do

Time in culture (days)
Relative In Vivo age